analytical HPLC to allow rapid and effective identification of metabolites in urine, following the oral administration of GW420867. The combination of these techniques enables structural and molecular weight information to be interpreted with greater efficiency and accuracy.

## Future Prospects

The advantages of capillary HPLC and nanospray MS, both 'miniaturized' versions of their predecessors, HPLC and electrospray, are well documented and there is also a move towards miniaturization in most other modern analytical techniques. The enhanced sensitivity obtained by capillary LC using a capillary UV flow cell may be extended to NMR, with the development of capillary NMR flow cells. The higher efficiency separations and reduced band broadening that result from capillary LC has the effect of producing a more concentrated sample eluting from the column and therefore a higher sample concentration into the NMR flow cell. This potentially leads to a better signal to noise ratio at lower analyte concentrations in the acquisition of NMR data. A further advantage of capillary LC is the reduced solvent consumption associated with this technique, which can lead to particularly significant cost-savings when dealing with the expensive deuterated solvents used in NMR spectroscopy. Residual protonated solvent suppression in the NMR spectrum then becomes an easier task.

Capillary LC and capillary electrochromatography (CEC) coupled to mass spectrometry are already in widespread use within the pharmaceutical industry and therefore the connection of capillary LC or CEC to both NMR and MS would be a natural progression.

*See also:* **II/Chromatography: Liquid:** Detectors: Mass Spectrometry; Large-Scale Liquid Chromatography; Nuclear Magnetic Resonance Detectors.

## Further Reading

Chervet JP, Ursen M and Salzmann JP (1996) *Anal. Chem.* 68: 1507.

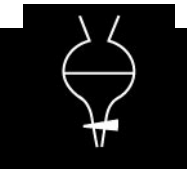
Olson DL, Lacey ME and Sweedler JV (1998) *Anal. Chemistry News and Features*, April.

Vanhoutte K, Van Dongen W and Esmans L (1998) *Rapid Commun. Mass Spectrom.*, 12: 15.

Vanhoutte K, Van Dongen W, Hoes I *et al.* (1997) *Anal. Chem.* 69: 3161.

DropBooks

# DRUGS OF ABUSE: SOLID-PHASE EXTRACTION



**F. Musshoff**, Institute of Legal Medicine, Bonn, Germany

Copyright © 2000 Academic Press

## Introduction

In toxicological analysis, two basic approaches can be distinguished: first, a directed search, geared to a limited number of substances, such as in workplace testing or the analysis of alcohol or special drugs in traffic offences; and second, an undirected search, also called systematic toxicological analysis (STA). STA can be defined as an undirected search for potentially harmful substances whose presence are uncertain and whose identities are unknown. STA is required if little or no information is available in so-called general unknown cases. However, if one toxicant is known, the analyst is required to establish whether other compounds of toxicological relevance are present.

The drug screening process can generally be divided into two stages, sample preparation and analysis of the drugs. Some forms of sample work-up – isolation and concentration – are required for most analytical techniques, such as thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC) coupled to various detector systems. The samples available for analysis are complex biological matrices, in which toxicologically relevant substances are present in trace amounts compared to the endogenous compounds present. Therefore, work-up procedures should retain all relevant substances, at the same time removing all irrelevant substances and interferences.

Liquid–liquid extraction (LLE), often combined with sample pretreatment procedures such as conjugate hydrolysis, digestion or protein removal, was the standard method in the past. Although LLE proved to be suitable in a great number of cases, there are many disadvantages of this technique, e.g. matrix interferences, emulsion formation or the use of large

**Table 1** Various solid-phase extraction adsorption phases and solvents

| Solubility of the sample | Water-soluble | | | | | Water-insoluble | | |
|---|---|---|---|---|---|---|---|---|
| Solvent | Nonionic Aqueous | | | Ionic Aqueous | | Aqueous | Organic | Organic |
| Polarity of the sample | Nonpolar | Middle polar | Polar | Cationic | Anionic | Nonpolar | Middle polar | Polar |
| Recommendable adsorption phase | $C_{18}$ec<br>$C_{18}$<br>$C_8$<br>$C_4$<br>$C_2$<br>Phenyl<br>CN | SiOH<br>$NH_2$ | CN<br>OH<br>PA<br>DMA<br>$NH_2$ | SA | SB<br>$NH_2$<br>DMA | $C_{18}$ec<br>$C_{18}$<br>$C_8$<br>$C_4$<br>$C_2$<br>Phenyl<br>CN | SiOH<br>$NH_2$ | CN<br>OH<br>PA<br>DMA<br>$NH_2$ |
| Selection of recommendable elution solvents | Hexane<br>$CH_2Cl_2$<br>Acetonitrile<br>Alcohols | $CHCl_3$<br>$CH_2Cl_2$<br>Ethyl acetate<br>Alcohols<br>Water | $CHCl_3$<br>$CH_2Cl_2$<br>Ethyl acetate<br>Alcohols<br>Water | Acids<br>Salt solutions<br>Buffers | Bases<br>Salt solutions<br>Buffers | Hexane<br>$CH_2Cl_2$<br>Acetonitrile<br>Alcohols | $CHCl_3$<br>$CH_2Cl_2$<br>Ethyl acetate<br>Alcohols | $CHCl_3$<br>$CH_2Cl_2$<br>Ethyl acetate<br>Alcohols |

volumes of organic solvents. In recent years, sample preparation by solid-phase extraction (SPE) has received widespread interest and today many types of SPE materials are commercially available (**Table 1**). Most publications have been geared towards the isolation of one compound or a limited number of substances, i.e. directed analysis. However, in STA (undirected analysis), a compromise between acceptable recovery of many substances and adequate removal of matrix compounds should be reached. In order to develop a SPE method, each step of the procedure should be evaluated very carefully, including the selection of a suitable sorbent, the pH of the sample and the extraction system, the clean-up step, the properties and the volume of the eluent, the flow rate of the sample and the eluent passed through column.

## Pretreatment of Biological Fluids and Tissues

The main purposes of sample pretreatment are first, release of drugs from the biological matrix; second, removal of proteins and other compounds which could interfere with further analysis; and third, adjustment of the pH, ionic strength and concentration of the sample to allow optimum extraction.

For the analysis of plasma/serum samples, buffer solutions are widely used for dilution. Protein precipitation is a common method to obtain deproteinized samples. Proteins can be precipitated by organic solvents, inorganic salts, metallic ions or acids (**Table 2**). However, it must be emphasized that co-precipitation often results in losses of the relevant drugs. Urine

**Table 2** Relative efficiencies of various protein precipitants towards removing proteins

| *Precipitant* | *pH of supernatant* | *Volume of precipitant (mL) to precipitate > 98% protein in 0.5 mL plasma* |
|---|---|---|
| Trichloroacetic acid, 10% (w/v) | 1.4–2.0 | 0.2 |
| Perchloric acid, 6% (w/v) | < 1.5 | 0.4 |
| Tungstic acid | 2.2–3.9 | 0.6 |
| Metaphosphoric acid, 5% | 1.6–2.7 | 0.6 |
| Copper sulfate–sodium tungstate | 5.7–7.3 | 1.5 |
| Zinc sulfate–sodium hydroxide | 6.5–7.5 | 2.0 |
| Zinc sulfate–barium hydroxide | 6.6–8.3 | 2.0 |
| Ammonium sulfate (saturated) | 7.0–7.7 | 2.0 |
| Acetonitrile | 8.5–9.5 | 1.5 |
| Acetone | 9.0–10.0 | 1.5 |
| Ethanol | 9.0–10.0 | 2.0 |
| Methanol | 8.5–9.5 | 2.0 |

Reprinted from Blanchard J (1981) Evaluation of the relative efficacy of various techniques for deproteinizing plasma samples prior to high-performance liquid chromatographic analysis. *Journal of Chromatography* 226: 455, with permission form Elsevier Science.

samples can usually be diluted like plasma/serum samples. However, since many relevant substances or metabolites will be excreted in a conjugated form, a deconjugation step prior to SPE is recommended. Because of the highly variable salt content, the urine samples should be diluted with at least an equal volume of water or buffer before applying the sample on to the SPE column. This is particularly important when ion exchange is the preferred extraction mode; otherwise counterions may compete with the relevant drugs during sample application and result in losses of the latter. Sonication combined with buffer dilution is a useful technique for the pretreatment of whole blood samples. For pretreating tissue samples, homogenization followed by enzyme digestion (i.e. papain, subtilisin-A, neutrase, collagenase or trypsin) and/or protein precipitation and centrifugation of samples such as liver, kidney or intestine prior to application on to SPE cartridges are useful. Brain tissue with a high content of lipids can be used after incubation with lipase prior to extraction. High flow SPE columns are available for use with more viscous fluids.

As in LLE, pH is an important factor in SPE. The optimal pH values of the sample and the extraction system depend on the properties of the relevant drugs and the sorbent and the interaction between the drugs and the functional groups of the sorbent. When a nonpolar sorbent, for example, octadecyl-bonded silica ($C_{18}$), is used, the main interactions are van der Waals forces/hydrogen bonding. Therefore, the pH of both sample and the column should be adjusted to a value so that the relevant drugs are in their uncharged forms. With ion exchange as the underlying principle, the pH of the sample must be adjusted to such a value that most of the drugs are charged, so that they can be retained on the column by the opposite charge of the functional groups of the sorbent. Additionally, total ionic strength of the sample is important in ion exchange SPE. A low ionic strength, often obtained by diluting the sample with water or a low ionic strength buffer solution ($< 0.1\ \text{mol L}^{-1}$) is preferred, because any species that can act as counterions reduces the retention of ionic drugs.

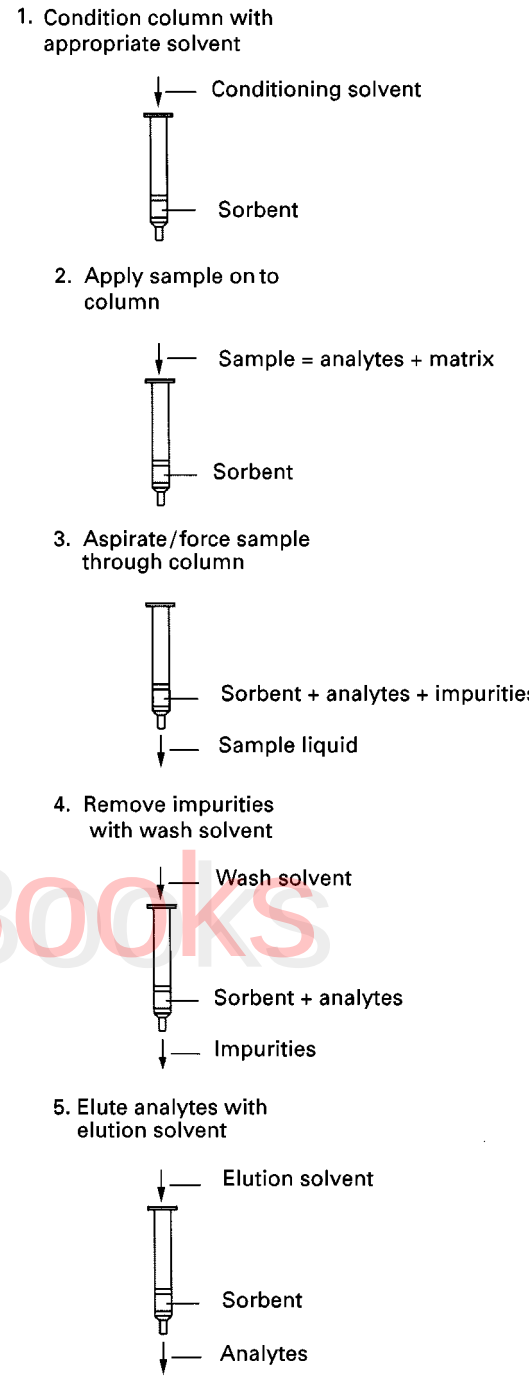



**Figure 1** Solid-phase extraction process.

## The Principles of Solid-phase Extraction

SPE is a physical extraction process that involves a solid phase and a liquid phase. It is based on the principle of liquid chromatography, but with different purposes. The aims of SPE are to isolate the relevant compounds from a sample matrix and to concentrate them, while those of liquid chromatography are to separate each compound with a good peak shape and with relatively short retention times. Today, various sorbent materials are commercially available. **Figure 1** illustrates the steps of SPE. The column is first conditioned with an appropriate solvent to solvate the functional groups of the sorbent. After the solvent is further conditioned with the sample matrix buffer, the pretreated sample is forced

through the sorbent by aspiration or positive pressure. The column containing retained analytes is subsequently washed with an appropriate solvent that selectively elutes the impurities but leaves the analytes on the column. The purified analytes are finally eluted with a solvent strong enough to displace the analytes from the sorbent.

## Basic Types of Solid Phases

### Diatomaceous Earth (Extrelut®, Chemelut®)

The principle of SPE using diatomaceous earth is closely related to conventional LLE. The aqueous phase is absorbed on to the diatomaceous earth, a porous material which acts as support for the aqueous phase. This provides a large surface area for partition into an elution solvent, which flows through the immobilized specimen on the column under gravity. The elution is a continuous process and may give superior recoveries in a shorter time compared to LLE. Other advantages are the elimination of centrifugation, aspiration and filtration steps and the prevention of emulsion formation. However, relatively large volumes of organic solvents are still required. For screening purposes this type of SPE must be carried out with at least two columns: one for acidic and neutral substances and one for basic and neutral compounds. A typical procedure with this type of material is as follows: the biological sample is diluted with an appropriate buffer and poured on to the column. The bed mass of the column and the sample volume must be in agreement with each other. After a 10–15 min equilibration period, twice the volume of the diluted aqueous sample is used for elution from an organic solvent, which is waterimmiscible.

### Styrene-divinylbenzene Resin (SDB)

Polystyrene-divinylbenzene copolymer (e.g. XAD-2) is a hydrophobic resin that can absorb many water-soluble organic compounds, principally by van der Waals forces and additionally by hydrophobic bonding and dipole–dipole interactions. For binding to the resin the substances must be in a hydrophobic state. Therefore, usually two columns are needed: one for acidic and neutral substances and one for basic and neutral compounds. Generally the extracts are clean enough to allow GC or TLC determinations at therapeutic and toxic concentrations. SDB resin is especially interesting for analysing urine samples since glucuronide and sulfate conjugates can be isolated. However, the extraction yields of drugs isolated from different biological samples may vary considerably. The resin has to be cleaned very carefully, otherwise interfering substances originating from the resin will appear in the extracts. SDB extractions in columns have now been largely replaced by SPE using silica-based columns. Recently, new SDB-based SPE columns (e.g. Bond Elut ENV, Varian) have become available, with which the drawbacks may be overcome. A typical procedure with this type of material is as follows: the biological sample is diluted and the pH is adjusted to the desired value. The resin is washed with four column volumes of acetone, three column volumes of methanol and three times with three column volumes of distilled water. The diluted sample is passed through the column where the analytes are absorbed. After the resin is washed with water, the analytes are eluted with an organic solvent (e.g. methanol, ethyl acetate, methanol–chloroform, acetone–diethyl ether, etc.).

### Octadecyl-bonded Silica

Octadecyl-bonded silica absorption phases (e.g. $C_{18}$-end-capped) are often used for the directed search to a limited number of substances, such as in testing for special drugs of abuse in traffic incidents. The nonpolar phase retains, at a suitable pH, substances by hydrophobic interactions with the alkyl chains. For example, the simultaneous analysis of tetrahydrocannabinol (THC) and its metabolites, 11-hydroxy-THC (11-OH-THC) and 11-nor-$\Delta^9$-THC carboxylic acid (THC-COOH) in serum samples is possible as follows: the biological sample is diluted with 0.01 mol $L^{-1}$ acetic acid and the pH is adjusted to 4. An organic solvent (methanol) is used to solvate the bonded functional groups and to remove organic residues from the sorbent. Buffer is added afterwards to exchange the organic solvent with an aqueous solution. The diluted sample is passed through the column where analytes absorb. After the column is washed with water followed by a solution of 40% acetonitrile in water, the analytes are eluted with acetonitrile (**Figure 2**).

### Mixed-mode Bonded Silica

The most widely used SPE materials are bonded silica gels, in which end silanol groups have been derivatized with organic moieties consisting of alkyl chains with and without a variety of functional groups, such as –OH, $-C_6H_5$, $-NH_2$, –CN, $-SO_3H$ and –COOH. Based on the modes of the interaction mechanisms between the functional groups of the SPE materials and the relevant compounds, the extraction can be divided into three types: nonpolar, polar and ion exchange. However, there is no bonded silica SPE column which only contains one type of functional

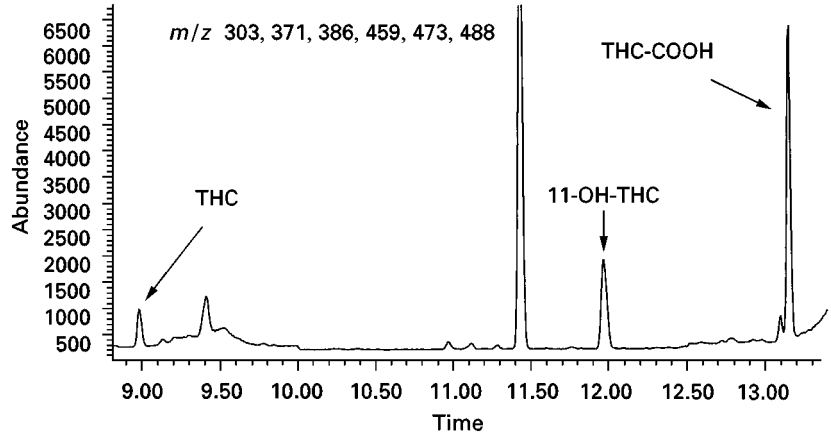



**Figure 2** GC-MS chromatogram recorded in the selected ion monitoring (SIM) mode. The serum sample was worked up using a $C_{18}$-end-capped extraction column and after derivatization of the dried extract using MSTFA after cannabinoids had been determined: THC (5.2 ng $mL^{-1}$), 11-hydroxy-THC (4.5 ng $mL^{-1}$) and THC-COOH (105.6 ng $mL^{-1}$).

group. Multiple modes of interactions will happen during the extraction process and the influence of secondary interactions should be kept in mind. Additionally, in so-called mixed-mode silica-bonded SPE columns, the silanol groups are partially derivatized with medium length alkyl chains and partially with cation exchange substituents, which can exert at least two types of interactions. Screening procedures using this type of SPE material have been of increasing interest and SPE columns with mixed-mode phases are available from a number of manufacturers, e.g. Bond Elut Certify (Varian Sample Preparation Products), Clean Screen DAU (Worldwide Monitoring Corp.), Isolute HCX (International Sorbent Technology) and TSC (Merck). Mixed-mode bonded silica can retain, at a suitable pH, acidic and neutral substances by hydrophobic interactions with the alkyl chains and the basic substances by interactions with the cation exchange groups. Differential elution can take place by a suitable adjustment of the pH and the choice of solvents.

A typical extraction procedure is described here in more detail.

**Sample pretreatment** Dilution with a 0.1 mol $L^{-1}$ phosphate buffer at pH 6.0 is most widely used. At this pH the weakly basic, the neutral and the weakly acidic compounds, such as barbiturates, are in the nonionized form and retained by the octadecyl substituent of the sorbent. However, strongly acidic compounds like many nonsteroidal anti-inflammatory drugs are deprotonated, ionized and therefore not retained. When blood samples are brought to lower pH values coagulation of proteins occurs, resulting in difficulties in the sample application step. When a serum or plasma sample is added to 0.1 mol $L^{-1}$ phosphoric acid, this coagulation can be avoided.

**Column preconditioning** The dried sorbents in SPE columns are not in a state to interact with analytes and appropriate conditioning is required prior to sample application. For nonpolar and multiple-interaction phases (mixed-mode), the sorbent must be preconditioned with suitable solvents, for example methanol, followed by water or a buffer wash. The organic solvent used is to solvate the bonded functional groups and to remove organic residues from the sorbent. Water or buffer, of which the pH, ionic strength and polarity have been adjusted, is added afterwards to remove the organic solvent with an aqueous solution to prepare the SPE column to receive an aqueous sample. For a polar phase, for example aminopropylsiloxane-bonded silica, the column needs to be treated with a nonpolar solvent, such as hexane, to activate the surface.

**Sample application** After column preconditioning, the pretreated sample is transferred onto the SPE column and is drawn through it by applying a light vacuum. Normally the flow rate of sample passing through the SPE cartridge should be kept to 1–2 mL $min^{-1}$.

**Column wash and pH adjustment** Usually the column is washed with 1–2 mL deionized water or an appropriate solvent (20% methanol in water) selectively to remove the impurities which may interfere with the analysis. To find a wash solvent which is

DropBooks

able to clean the column effectively without losses of the drugs, the analyst must compromise between acceptable recoveries of a great number of different substances and adequate removal of matrix compounds. In many cases, pH adjustment is introduced into this stage to bring the pH of the column to a given value for selective, pH-dependent elution of the drugs. In order to get a reproducible differential elution using mixed-mode bonded silica, the pH of the column has to be adjusted to about pH 3. At higher pH values a large number of basic compounds will elute in the first fraction (acidic and neutral substances). Lower pH values can cause deterioration of the extraction column. To adjust pH, 0.5–1.0 mL diluted acetic acid is sufficient.

**Column drying** Drying of the columns is necessary when no water is allowed in the analysing step (GC). Drying is carried out by applying vacuum to the column for about 5 min or centrifugation of the column. Further drying can be carried out by applying a small volume of methanol (50 μL) or a larger volume of hexane (1 mL) followed by vacuum. A dry column is easily obtained, but there is a risk of partially eluting hydrophobic substances such as benzodiazepines in this wash process.

**Elution of relevant drugs** For drug elution, the eluent should be strong enough so that the drugs can be eluted completely with a reasonably small eluent volume. Furthermore, the eluent should be selective, so that interfering compounds will not be eluted together with the relevant drugs. Theoretically, eluent selection may be achieved by considering the polarity index ($P'$), the solvent selectivity and the eluotropic strength ($\varepsilon^0$) of the solution solvents. The strength of a solvent is its ability preferentially to dissolve compounds according to polarity, while the selectivity is its ability selectively to dissolve one compound as opposed to another. Solvents have been classified into eight selectivity groups according to their proton donor, proton acceptor and dipole interaction characteristics. **Figure 3** represents the properties of various solvent groups with different selectivities. For example, solvents in group I are strong proton acceptors/weak donors with intermediate dipole moments and solvents in group VIII are relatively strong donors/weak proton acceptors with virtually no dipole interactions.

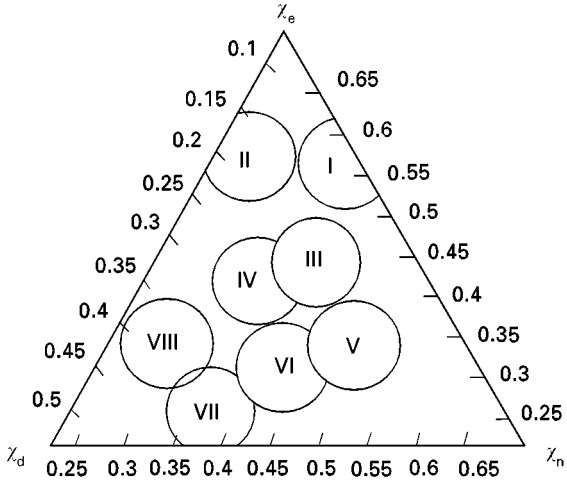



**Figure 3** Classification of solvent selectivities (the Roman numbers represent various groups of solvent selectivities). $\chi_e$, $\chi_d$ and $\chi_n$ represent the fraction of $P'$ contributed by interactions associated with ethanol (acceptor), dioxane (donor) and nitromethane (polar). Modified with permission from Snyder LR (1974) *Journal of Chromatography* 92: 223.

The $P'$ values and the selectivities of common solvents used in SPE are listed in **Table 3**. The desired $P'$ value can be obtained by using a binary mixture and can be calculated by the following equation:

$$P' = \phi_a P_a + \phi_b P_b \qquad [1]$$

where $\phi_a$ and $\phi_b$ are the volume fractions of solvents $A$ and $B$ in the mixture; $P_a$ and $P_b$ are the $P'$ values of the pure solvents $A$ and $B$. In practice, both $P'$ and selectivity of the solvents should be considered when selecting the best eluent system to elute drugs but not the impurities. Data shown in Table 3 indicate that the $P'$ values of 2-propanol, chloroform and ethyl acetate are very similar, yet they belong to different selectivity groups.

The eluotropic strength $\varepsilon^0$, which defines solvent strength quantitatively for a given adsorbent, is another helpful parameter for chosing a suitable eluent. The eluotropic strength is the adsorption energy per unit area of the solvent and Table 3 lists the $\varepsilon^0$ values of some common solvents and binary mixtures. This knowledge can be helpful in the development of a new SPE method. If a certain eluent system gives high recoveries of test drugs but also elutes many impurities, the use of another eluent mixture with a similar $\varepsilon^0$ value could be helpful.

The volume of a selected eluent is another important factor in the development of an extraction procedure. Generally, the volume of the eluent should be as small as possible. Increasing volumes will prolong the extraction period, may elute more impurities and may lose more volatile drugs (such as amphetamines) when an evaporation step is required after column extraction. The flow rate of the eluent passing through the column should allow adequate interac-

**Table 3** Solvent eluotropic strength on silica ($\varepsilon^0$), polarity indices ($P'$) (measure of solvent's ability to interact as proton donor, proton acceptor or dipole) and selectivities of some common solvents

| *Solvent* | *Eluotropic strength ($\varepsilon^0$)* | *Polarity ($P'$)* | *Selectivity group* |
|---|---|---|---|
| Water | > 0.73 | 10.20 | VIII |
| Acetic acid | > 0.73 | 6.20 | IV |
| Methanol | > 0.73 | 6.60 | II |
| Methanol–acetonitrile (40 : 60) | 0.67 | | |
| Methanol–diethyl ether (20 : 80) | 0.65 | | |
| 2-Propanol | 0.63 | 4.30 | II |
| Methanol–methylene chloride (20 : 80) | 0.63 | | |
| Pyridine | 0.55 | 5.30 | III |
| Isobutyl alcohol | 0.54 | 3.00 | |
| Acetonitrile | 0.50 | 6.20 | VI |
| Diethyl ether–acetonitrile (80 : 20) | 0.45 | | |
| Ethyl acetate | 0.45 | 4.30 | VI |
| Acetone | 0.43 | 5.40 | VI |
| Methyl ethyl ketone | 0.39 | 4.50 | VI |
| Tetrahydrofuran | 0.35 | 4.20 | III |
| Methylene chloride | 0.32 | 3.40 | V |
| Chloroform | 0.31 | 4.40 | VIII |
| Diethyl ether | 0.29 | 2.90 | I |
| Benzene | 0.27 | 3.00 | VI |
| Toluene | 0.22 | 2.40 | VI |
| Pentane–diethyl ether (80 : 20) | 0.20 | | |
| Cyclohexane | 0.03 | 0.00 | |
| Pentane | 0.00 | 0.00 | |
| *n*-Hexane | 0.00 | 0.06 | |
| *n*-Heptane | 0.00 | 0.20 | |

DropBooks

tion. When ion exchange is the main interaction, the flow rate should be $< 2$ mL min$^{-1}$ since ion exchange interactions occur at a slower rate than polar and nonpolar interactions.

Using mixed-mode bonded silica extraction columns in the first fraction, fraction *A*, the analytes retained by the hydrophobic groups of the sorbent are eluted using a moderately polar solvent like dichloromethane or combinations, e.g. acetone–chloroform (1 : 1), hexane–diethyl ether (40 : 60), hexane–ethyl acetate (75 : 25). To avoid dirty extracts in the second fraction, fraction *B* (basic compounds), an in-between polar washing step, for instance with methanol, may be needed.

The basic substances retained by the cation exchange groups of the sorbent in their protonated form are eluted by an organic solvent mixture, usually with 2% ammonia. Ammoniated ethyl acetate or ammoniated dichloromethane–2-propanol (80 : 20) for the elution of more polar substances is widely used. **Table 4** gives an overview of extraction methods using mixed-mode SPE phases for drug screening. Amphetamines and other relatively volatile substances often show lower recoveries, probably caused by evaporation in the final step of the SPE procedure. Polar drugs like acids and acetaminophenes are scarcely retained under the condition used and may be washed away. Therefore, an additional LLE on the sample coming from the column and column wash could be introduced in a general screening procedure.

## Conclusions and Further Developments

The use of SPE for the toxicological analysis of drugs of abuse in biological samples has increased rapidly. Due to the different properties of the drugs of interest, mixed-mode SPE columns are better suited for screening purposes than single-mode columns. However, although the same type of SPE material can be obtained from different manufacturers, the results using different materials, and even results obtained from different batches from the same manufacturer, may show significant differences in behaviour, i.e. in particle size distribution and flow velocities. Today, chemically modified silica and SDB sorbents are also available in extraction discs. These materials are very promising, since samples can be processed faster using smaller volumes of organic solvents while still allowing relatively large sample volumes. Furthermore, extractions can be performed outside working hours by automation of manual SPE methods with the addi-

**Table 4** Overview of mixed-mode SPE methods for drug screening

| *Sample type* | *SPE column type* | *Sample volume* | *Drug conc. ($\mu g\ mL^{-1}$ or $\mu g\ g^{-1}$)* | *Elution method (fraction A and fraction B)* | *Detection yield (%)* | *Extraction (%)* | *Relative standard deviation* |
|---|---|---|---|---|---|---|---|
| Urine (U) Plasma (P) | CS DAU | A: 4 mL U B: 5 mL U | 0.5–2 | A: 10 mL DCM B: DCM–2PrOH–25% $NH_3$ (147 : 49 : 4) | TLC GC-MS | 61–88[a] | < 9 |
| | BEC | 2 mL U/P | 10 | A: 4 mL Clf–Ac (1 : 1) B: 2 mL EtAc–33% $NH_3$ (98 : 2) | GC-FID | 97–104 | < 6 |
| | BEC | 1 mL U | 0.05 | A: 1 mL Hex–ETAC (8 : 2) B: 2 mL DCM–2PrOH–25% $NH_3$ (80 : 20 : 2) | GC-MS | | |
| | 1: BEC 2: Isolute | 5 mL U | 0.4–1 | A: 3 mL Hex–EtAc (75 : 25) B: 3 mL EtAc–28% $NH_3$ (98 : 2) | GC-MS | 1: 60–88[b] 2: 48–88[b] | 1: < 10 2: < 8 |
| | BEC | 1 mL U/P | 0.1–0.2 | A: 4 mL Clf–Ac (1 : 1) B: 2 mL EtAc–33% $NH_3$ (98 : 2) | GC-NPD | U: 82–105 P: 77–103 | U: < 8 P: < 7 |
| Whole blood | BEC | 1 mL | 0.05–5 | A: 4 mL DCM B: 4 mL EtAc–25% $NH_3$ (98 : 2) | GC-FID | 25–104[c] | < 14 |
| | BEC | 1 mL | 2 | A: 4 mL Clf–Ac (1 : 1) B: 2 mL EtAc–33% $NH_3$ (98 : 2) | GC-FID | 81–103 | < 8 |
| | BEC | 1 mL | 0.2–4 | A: 2 mL 60% acetone[d] B: 2 mL DCM–2PrOH–25% $NH_3$ (80 : 20 : 2) | GC-NPD | 50–100 | < 8 |
| | 1: BEC 2: CS DAU | 1 mL | 1 | A: 3 mL Hex–EtAc (1 : 1) B: 3 mL DCM–2PrOH–28% $NH_3$ (78 : 20 : 2) | GC-NPD | 1: 73–112 2: 59–115 | 1 : 9.7[e] 2 : 7.8 |
| | BEC | 1 mL | 0.5 | A: 4 mL Clf–Ac (1 : 1) B: 2 mL EtAc–25% $NH_3$ (98 : 2) C: 2 mL DCM–2PrOH–25% $NH_3$ (80 : 20 : 2) | GC-MS[f] | | |
| | BEC | 1 mL | 0.05–0.5 | A: 2 mL Clf–Ac (1 : 1) B: 3 mL EtAc–33% $NH_3$ (98 : 2) | GC-NPD GC-MS[f] | 58–107[d] 26–117 | < 11 < 16 |
| Tissue | XTRACT | 1.25 g | | A: 2 mL DCM; 2 mL Hex–Eth (4 : 6) B: 4 mL DCM–2PrOH–25% $NH_3$ (80 : 20 : 2); 4 mL EtAc | GC-MS | | |
| | BEC | 0.1 g | 20 | 4 mL Clf–Ac (1 : 1) B: 2 mL EtAc, 33% $NH_3$ (98 : 2) | GC-NPD GC-FID | 45–101 | < 9 |

**SPE column materials**: CS DAU: Clean screen DAU, Worldwide Monitoring, Horsham, PA.
BEC: Bond Elut Certify, Varian Sample Preparation Products, Harbor City, CA.
Isolute: Isolute HCX, International Sorbent Technology, Hengoed Mid Glamorgan, UK.
XTRACT: Worldwide Monitoring, Horsham, PA.

**Abbreviations** AC, acetone; Clf, chloroform; DCM, dichloromethane; EtAc, ethyl acetate; Eth, diethyl ether; Hex, hexane; $NH_3$, concentrated ammonia; 2PrOH, 2-propyl alcohol.

[a]One SPE column is used for acidic and neutral drugs and one for basic drugs.
[b]Low recoveries for barbital and ephedrine.
[c]Morphine and amphetamine are hardly recovered.
[d]Basic fractions of SPE were cleaned up by liquid–liquid extraction with butyl acetate; recovery of paracetamol is low.
[e]Mean values.
[f]TMS derivatization.
[g]Extraction yields at a spiked concentration of respectively 0.1 and 0.25 $\mu g\ mL^{-1}$.
Reproduced from Franke and de Zeeuw (1998) with permission.

tional intention of improving the reproducibility, offering high throughput, and reducing labour costs. Various automated SPE systems are commercially available.

*See also:* **II/Extraction:** Solid-Phase Extractions. **III/Solid-Phase Extraction with Cartridges. Sorbent Selection for Solid-Phase Extraction**.

## Further Reading

Chen XH, Franke JP and de Zeeuw RA (1992) Solid-phase extraction for systematic toxicological analysis. *Forensic Science Review* 4: 147.

Chen XH, Wijsbeek J, Franke JP and de Zeeuw RA (1992) A single-column procedure on Bond Elut Certify for systematic toxicological analysis of drugs in plasma and urine. *Journal of Forensic Sciences* 37: 61.

Ferrara SD, Tedeschi L, Frison G and Castagna F (1992) Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine. *Journal of Analytical Toxicology* 16: 217.

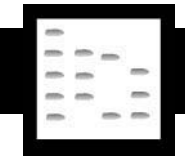
Franke JP and de Zeeuw RA (1998) Solid-phase extraction procedures in systematic toxicological analysis (review). *Journal of Chromatography* 713: 51.

Scheurer J and Moore CM (1992) Solid-phase extraction of drugs from biological tissues – a review. *Journal of Analytical Toxicology* 16: 264.

Van Horne KC (1985) *Sorbent Extraction Technology*. Harbor City, CA: Analytichem International.

Zief M and Kiser R (1988) *Solid Phase Extraction for Sample Preparation*. Phillipsburg, NJ: JT Baker.

# DYES



## High-Speed Countercurrent Chromatography

**A. Weisz**, Food and Drug Administration, Washington DC, USA
**Y. Ito**, National Institutes of Health, Bethesda, MD, USA

Copyright © 2000 Academic Press

Countercurrent chromatography is one of the liquid–liquid partition chromatographic techniques that do not use a solid support. High-speed countercurrent chromatography (HSCCC) uses centrifugal force to retain one of the liquid phases in an lto multilayered coil column while the second liquid phase is pumped through the column. The principles of this technique have been discussed by Ito.

HSCCC in its two forms, conventional and pH-zone-refining CCC, is relatively new among the preparative techniques used for the separation of dyes. Conventional HSCCC has been applied to this purpose since the mid-1980s when Fales *et al*. separated various components present in a sample of the triphenylmethane biological stain, Methyl Violet 2B, and when Freeman and co-workers purified azo textile and ink dyes (i.e. acid, direct and disperse azodyes). This technique was subsequently used for the separation and purification of components from other colours, such as D&C Red No. 28 (Phloxine B, CI 45410), Sulforhodamine B (CI 45100) and Gardenia Yellow. It was also effectively implemented as a complement to preparative high performance liquid chromatography (HPLC) for the separation of a complex synthetic mixture of brominated tetrachlorofluorescein dyes. Conventional HSCCC was applied to the separation of quantities of dyes of up to several hundred milligrams when the common 1.6 mm i.d./325 mL volume column was used. By contrast, the more recently developed (1993) pH-zone-refining CCC was applied from the outset to the separation of multi-gram quantities of dye mixtures such as xanthene and fluoran dyes used as colour additives in food, drugs or cosmetics and as biological stains.

Using a modified procedure, the applications of this technique have been extended to the separation of gram quantities of the highly polar mono-, di- and trisulfonated components of D&C Yellow No. 10 and Yellow No. 203 (both Quinoline Yellow, CI 47005) and of other sulfonated dyes such as FD&C Yellow No. 6 (Sunset Yellow, CI 15985) and D&C Green No. 8 (Pyranine Concentrated, CI 59040). A general approach to the separation of dyes by HSCCC is presented in **Figure 1**.

DropBooks

### Instrumentation

For the separation of dyes, both by conventional and pH-zone-refining CCC, commercially available high speed CCC centrifuges are used. Such instruments are described in the HSCCC entry in this volume. The separations described below were performed with HSCCC systems from PC, Potomac, MD, USA and Pharma-Tech Research, Baltimore, MD, USA. A schematic diagram of a HSCCC system used for separation of dyes is shown in **Figure 2**.

## Conventional HSCCC

Conventional HSCCC may be applied only to the separation of relatively small amounts (up to several hundred milligrams when the common 325 mL volume column is used) of dye mixtures. In contrast to pH-zone-refining CCC, conventional HSCCC also permits separation of nonionic components. Analytical size separations (less than 10–20 mg of an ionic or nonionic component of interest) should be performed by conventional HSCCC.

#### Selection of the Solvent System

The solvent system used for a conventional HSCCC separation is selected according to the hydrophobicity